Supplementary Material.

Estimations of Human Equivalent Exposure.

The human equivalent exposure time periods required for acquiring an equivalent deposited dose as employed in this study were estimated – see below. Specifically, human equivalent workplace exposure to a deposited dose of 18μg of total carbon can be achieved in ~156.25 working days at allowable exposure concentration limits defined by MSHA (160 $\mu g/m^3$ of total carbon). In addition to justifying the particle concentrations used in this study, this suggests humans can be exposed chronically for longer times to significantly higher doses of D or BD. Notably, subpopulations of humans may exert elevated susceptibility to the effects of biodiesel or diesel particulates, thus justifying the need in even higher experimental particulates concentrations in animal models.

<u>Given that :</u>

MSHA Conc. Of allowable exposure to exhaust (A) = 160 $\mu g/m^3$ of total carbon
Minute volume (B) = 20 L/min = 20000 ml/min
Deposition efficiency of particles of size ~250nm (C) = 15%
Total alveolar surface area of human lung (D) = 120 m^2
Total alveolar surface area of mouse lung (E) = 0.06 m^2
Dosage used in mice = 18 μg of TC/mouse
= 18 μg/0.06 (total of mouse lung in m^2) = 300 $\mu g/m^2$

<u>Equivalent Exposure in humans :</u>

300 $\mu g/m^2$ in mice = (A x B x C x duration)/D
Exposure duration in humans = (300 x D)/(A x B x C)
= (300 x 120)/(160 $\mu g/m^3$ x (20000 ml/min * 10^{-6} m^3/ml) x 0.15)
= (300 x 120) / 0.48 min
= 75000 min = (1250 hours)/8 hours per day = 156.25 working days

Supplementary Figures.

Figure S1: The photographs of the (A) VACES system at NIOSH OMSHR DL, and (B) BioSampler with DPM sample.

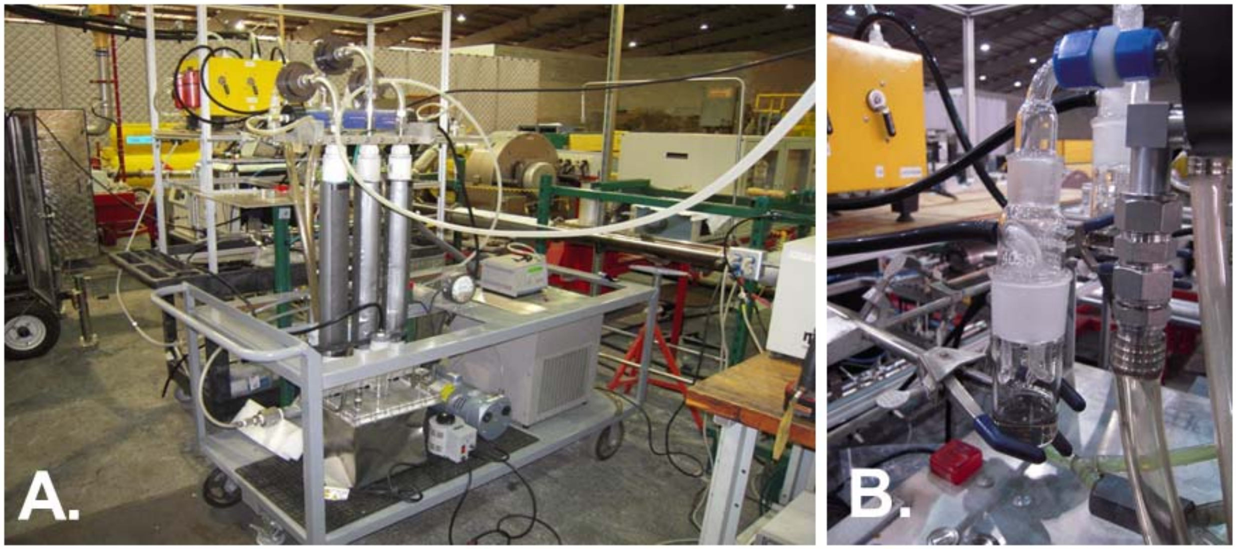


Figure S2: Distribution profiles of exhaust particles from neat (A) BD and (B) D as determined using dynamic light scattering.

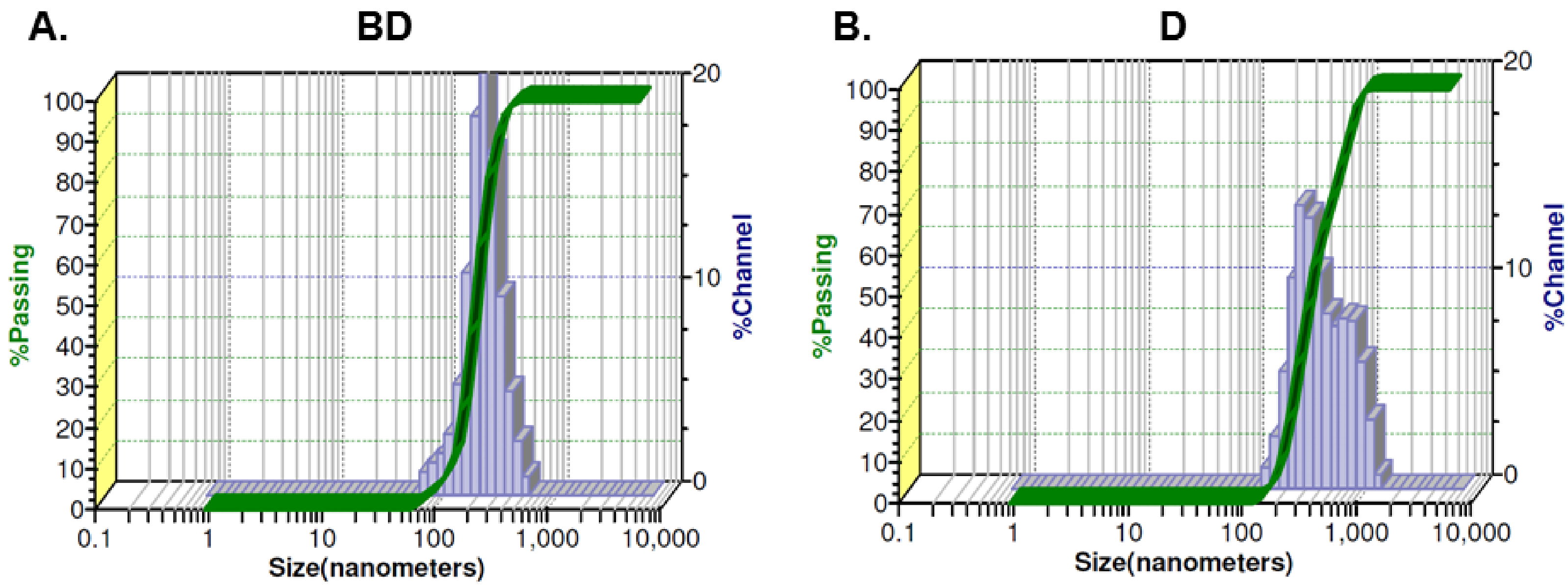
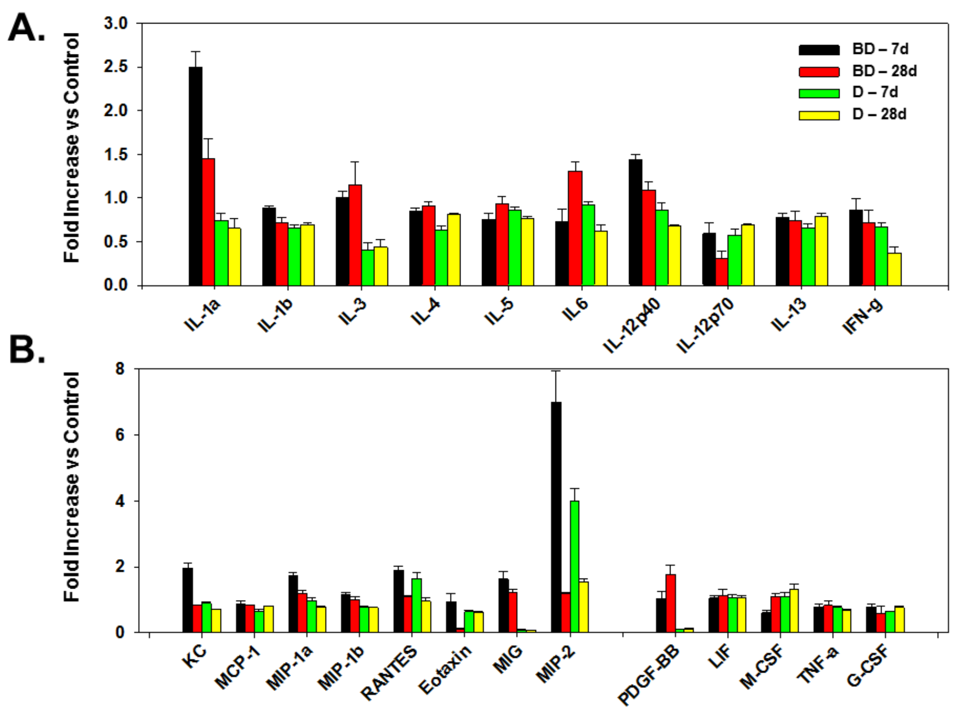


Figure S3: Accumulation of cytokines, chemokines and growth factors at 7 and 28 days post exposure to neat BD and D exhaust particles. Comparison of the levels of inflammatory cytokines (A), chemokines and growth factors (B) in the lung homogenates and BAL fluid of C57BL/6 mice (n = 5) following aspiration of 18 μg/mouse of total carbon or 100 μg/mouse of BD or D exhaust particles. These measurements were performed using Bio-rad 23-plex and 9-plex mouse assay kits, composed of a combination of pro- and anti- inflammatory cytokine along with a sub set of chemokine's and growth factors. The data are represented as means ± SE of fold increase compared to controls in each case. The break in the chemokine/growth factors data in the BAL fluid indicates that the scale representing from 120-1000 has been omitted from the graph.



Supplementary Tables.

Table S1: Pre-combustion components of Biofuels from various sources presented as percentage of major saturated, mono- and poly-unsaturated free fatty acids in several types of feedstock used for Biodiesel fuel.

Biodiesel Source	**12:0**	**14:0**	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**	**20:0**	**20:1**	**22:1**
Soybean	.	.	6-10	2-5	20-30	50-60	5-11	.	.	.
Corn	.	1-2	8-12	2-5	19-49	34-62	.	.	.	.
Peanut	.	.	8-9	2-3	50-65	20-30	.	.	.	.
Olive	.	.	9-10	2-3	73-84	10-12	.	.	.	.
Cottonseed	.	0-2	20-25	1-2	23-35	40-50	.	.	.	.
Linseed Oil	.	.	4-7	2-4	25-40	35-40	25-60	.	.	.
Coconut Oil	45-53	17-21	7-10	2-4	5-10	1-3	.	.	.	.
Palm oil	.	.	44	5	39	10	.	.	.	.

Table S2: The specifications of Isuzu C240 engine. The engine is coupled to a water-cooled eddy-current dynamometer from SAJ (Pune, India, Model SE150) rated at 150 kW (201 bhp).

Isuzu C240		Unit
Type	in-line 4	-
Cycles	4	-
Cooling	Water	-
Valves	Overhead	-
Injection	Indirect	-
Air intake system	naturally aspirated	-
Engine management	mechanical	
Displacement	2369 (145)	cm^3 (in^3)
Intermittent rating	41.8 (56) @ 3000 rpm	kW (bhp)
Continuous rating	36.5 (49) @ 3000 rpm	kW (bhp)
Peak torque	146.4 (108) @ 2000 rpm	Nm (lb ft)
Moment of inertia	1499 (35.59)	kg m^3 (lb ft^2)
Engine speed at idle	700±50	Rpm
Maximum engine speed at no load	3260±50	Rpm

Table S3: Details of engine operating conditions.

Mode	Description	Engine Speed	Torque	Power
		rpm	Nm	kW
R50	Rated speed 50% load	2950	55.6	17.2
R100	Rated speed 100% load	2950	111.2	34.3
I50	Intermediate speed 50% load	2100	69.1	14.9
I100	Intermediate speed 100% load	2100	136.9	30.6

Table S4: Accumulation of Cytokines in the lung homogenates and BAL fluid of mice following 24h, 7d and 28d post pharyngeal aspiration with neat BD and D exhaust particles. The data is represented as fold increase vs control (mean ± SEM) as is in BAL fluid and in pg/mg of total protein observed in tissue homogenates. The cytokines that were up-regulated (>1.5 fold vs control) upon B100 and D100 exposure in lung tissue homogenates and BAL fluid are highlighted in bold and colored in red and blue, respectively.

Sample Type	Diesel Type	End Points	IL-1α	IL-1β	IL-3	IL-4	IL-5	IL6	IL-12p40	IL-12p70	IL-13	IFN-γ
Pulmonary Tissue Homogenate	B100	control	1 ± 0.17	1 ± 0.07	1 ± 0.06	1 ± 0.04	1 ± 0.09	1 ± 0.17	1 ± 0.02	1 ± 0.1	1 ± 0.07	1 ± 0.05
		24h	**68.91 ± 13.06**	**6.41 ± 0.8**	**1.36 ± 0.08**	**1.6 ± 0.13**	0.97 ± 0.02	**3.77 ± 1.04**	**3 ± 0.3**	**2.4 ± 0.01**	0.77 ± 0.07	**1.49 ± 0.02**
		7d	**2.49 ± 0.18**	0.89 ± 0.03	1 ± 0.08	0.85 ± 0.02	0.76 ± 0.07	0.73 ± 0.15	1.44 ± 0.05	0.59 ± 0.13	0.77 ± 0.06	0.86 ± 0.14
		28d	**1.45 ± 0.23**	0.71 ± 0.07	1.15 ± 0.26	0.91 ± 0.04	0.94 ± 0.08	1.31 ± 0.11	1.09 ± 0.09	0.31 ± 0.08	0.74 ± 0.1	0.71 ± 0.14
	D100	control	1 ± 0.07	1 ± 0.06	1 ± 0.14	1 ± 0.12	1 ± 0.12	1 ± 0.06	1 ± 0.07	1 ± 0.14	1 ± 0.14	1 ± 0.21
		24h	**4.29 ± 0.31**	1.01 ± 0.04	0.96 ± 0.14	0.74 ± 0.03	0.76 ± 0.02	0.94 ± 0.09	1.28 ± 0.16	0.64 ± 0.03	0.66 ± 0.02	0.97 ± 0.14
		7d	0.74 ± 0.08	0.65 ± 0.04	0.4 ± 0.09	0.63 ± 0.05	0.86 ± 0.04	0.92 ± 0.04	0.86 ± 0.09	0.58 ± 0.07	0.65 ± 0.05	0.66 ± 0.05
		28d	0.65 ± 0.12	0.69 ± 0.03	0.44 ± 0.08	0.81 ± 0.01	0.77 ± 0.02	0.62 ± 0.07	0.68 ± 0.02	0.69 ± 0.01	0.79 ± 0.04	0.37 ± 0.07
BAL Fluid	B100	control	1 ± 0.01	1 ± 0.18	1 ± 0.1	1 ± 0.01	1 ± 0.33	1 ± 0.21	1 ± 0.14	1 ± 0.28	1 ± 0.21	1 ± 0.68
		24h	**12.13 ± 0.34**	1.39 ± 0.08	0.95 ± 0.09	**1.54 ± 0.21**	**2.81 ± 0.82**	**102.58 ± 14.71**	**5.47 ± 0.96**	0.97 ± 0.08	**2.35 ± 0.26**	0.89 ± 0.07
		7d	1.1 ± 0.03	1.16 ± 0.01	0.97 ± 0.02	1.18 ± 0.06	0.92 ± 0.03	1.03 ± 0.06	1.36 ± 0.16	0.79 ± 0.05	0.98 ± 0.01	1.08 ± 0
		28d	0.9 ± 0.03	1.01 ± 0.04	0.92 ± 0.04	1.02 ± 0.04	1.05 ± 0.01	0.94 ± 0.01	1.07 ± 0	1.1 ± 0.01	1.1 ± 0	1.11 ± 0
	D100	control	1 ± 0.34	1 ± 0.02	1 ± 0	1 ± 0.01	1 ± 0.08	1 ± 0.06	1 ± 0.02	1 ± 0.01	1 ± 0.1	1 ± 0.03
		24h	**2.17 ± 0.17**	1.19 ± 0.01	1.19 ± 0.01	1.3 ± 0.03	**3.36 ± 0.62**	**14.33 ± 1.33**	**5.59 ± 1.36**	0.99 ± 0.04	1.2 ± 0.04	0.9 ± 0.02
		7d	1.14 ± 0.03	1.11 ± 0.04	0.85 ± 0.03	1.16 ± 0.04	0.94 ± 0.02	0.95 ± 0.04	0.88 ± 0.03	0.81 ± 0.06	0.99 ± 0.01	1.01 ± 0.02
		28d	0.93 ± 0.01	0.96 ± 0	0.93 ± 0	1.02 ± 0.02	0.94 ± 0.01	0.99 ± 0	0.72 ± 0.02	1.08 ± 0.04	1 ± 0.02	1.04 ± 0

Table S5: Accumulation of chemokines and growth factors in the lungs of mice exposed to neat BD and D exhaust particles. The data is represented as fold increase vs control (mean ± SEM) as is in BAL fluid and in pg/mg of total protein observed in tissue homogenates. The chemokines and growth factors that were up-regulated (>1.5 fold vs control) upon B100 and D100 exposure in lung tissue homogenates and BAL fluid are highlighted in bold and colored in red and blue, respectively.

Sample Type	Diesel Type	End Points	Chemokines								GrowthFactors				
			KC	MCP-1 (MCAF)	MIP-1a	MIP-1b	RANTES	Eotaxin	MIG	MIP-2	PDGF-BB	LIF	M-CSF	TNF-a	G-CSF
Pulmonary Tissue Homogenate	B100	control	1 ± 0.06	1 ± 0.06	1 ± 0.05	1 ± 0.04	1 ± 0.06	1 ± 0.2	1 ± 0.12	1 ± 0.07	1 ± 0.24	1 ± 0.13	1 ± 0.11	1 ± 0.07	1 ± 0.12
		24h	**36.56 ± 0.54**	**7.01 ± 1.5**	**31.91 ± 12.66**	**4.45 ± 0.18**	**4.04 ± 0.29**	**1.75 ± 0.29**	**4.04 ± 0.97**	**147.95 ± 23.02**	0.87 ± 0.12	**3.19 ± 0.58**	**3.96 ± 1.02**	1.23 ± 0.03	**53.7 ± 6.91**
		7d	**1.97 ± 0.14**	0.89 ± 0.08	**1.74 ± 0.09**	1.14 ± 0.07	**1.91 ± 0.12**	0.94 ± 0.25	**1.62 ± 0.25**	**6.99 ± 0.97**	1.04 ± 0.2	1.05 ± 0.07	0.6 ± 0.08	0.78 ± 0.1	0.78 ± 0.09
		28d	0.84 ± 0.01	0.82 ± 0.02	1.2 ± 0.09	1.01 ± 0.1	1.1 ± 0.03	0.1 ± 0.06	1.23 ± 0.08	1.19 ± 0.05	**1.76 ± 0.28**	1.13 ± 0.18	1.11 ± 0.09	0.82 ± 0.13	0.57 ± 0.23
	D100	control	1 ± 0.14	1 ± 0.14	1 ± 0.05	1 ± 0.1	1 ± 0.14	1 ± 0.07	1 ± 0.43	1 ± 0.37	1 ± 0.69	1 ± 0.1	1 ± 0.11	1 ± 0.12	1 ± 0.14
		24h	**4.38 ± 1.11**	1.2 ± 0.07	**2.89 ± 0.29**	0.92 ± 0.01	**4.11 ± 0.61**	0.66 ± 0.03	0.12 ± 0.04	**33.35 ± 2.61**	0.1 ± 0	**1.73 ± 0.1**	**2.28 ± 0.12**	0.77 ± 0.05	**4.04 ± 0.92**
		7d	0.9 ± 0.05	0.66 ± 0.06	0.98 ± 0.08	0.76 ± 0.04	**1.63 ± 0.2**	0.64 ± 0.04	0.09 ± 0.02	**3.98 ± 0.4**	0.1 ± 0	1.08 ± 0.08	1.08 ± 0.12	0.76 ± 0.03	0.63 ± 0.02
		28d	0.72 ± 0.01	0.81 ± 0.01	0.79 ± 0.03	0.77 ± 0.01	0.95 ± 0.12	0.61 ± 0.05	0.06 ± 0	**1.55 ± 0.08**	0.12 ± 0.02	1.06 ± 0.06	1.3 ± 0.17	0.69 ± 0.04	0.77 ± 0.04
BAL Fluid	B100	control	1 ± 0.2	1 ± 0.33	1 ± 0.71	1 ± 0.11	1 ± 0.1	1 ± 0.28	ND	ND	ND	ND	ND	1 ± 0.01	1 ± 0.13
		24h	**20.9 ± 2.86**	**10.97 ± 3.11**	**21.16 ± 4.53**	**1.77 ± 0.45**	**1236.23 ± 535.03**	1.06 ± 0.07	ND	ND	ND	ND	ND	**2.11 ± 0.17**	**2108.46 ± 620.73**
		7d	**5.84 ± 0.96**	1.13 ± 0.03	**1.79 ± 0.13**	1.1 ± 0.03	**2.01 ± 0.27**	0.95 ± 0.02	ND	ND	ND	ND	ND	0.98 ± 0.02	1 ± 0.01
		28d	0.92 ± 0.08	1.02 ± 0.02	1.03 ± 0.06	1.04 ± 0.01	1.11 ± 0.05	1.06 ± 0.01	ND	ND	ND	ND	ND	0.96 ± 0.01	1.04 ± 0
	D100	control	1 ± 0.07	1 ± 0.01	1 ± 0.01	1 ± 0.17	1 ± 0.12	1 ± 0.09	ND	ND	ND	ND	ND	1 ± 0.08	1 ± 0.01
		24h	**10.24 ± 3.35**	**7.46 ± 0.62**	**2.47 ± 0.09**	**8.73 ± 0.98**	**25.47 ± 6.9**	0.87 ± 0.02	ND	ND	ND	ND	ND	1.16 ± 0.06	**101.96 ± 8.33**
		7d	**2.46 ± 0.64**	1.05 ± 0.03	**1.51 ± 0.04**	1.01 ± 0.03	**1.57 ± 0.24**	0.95 ± 0.03	ND	ND	ND	ND	ND	0.94 ± 0.02	0.95 ± 0.04
		28d	0.91 ± 0.03	0.97 ± 0.03	0.91 ± 0	1 ± 0	0.91 ± 0.02	1.07 ± 0	ND	ND	ND	ND	ND	0.95 ± 0	0.96 ± 0.01